

Colony PCR CYC series 1

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|---|---------------------------------|---------------------|--|------------|-------------------------|
| | PCR 20 µL reaction (units : µL) | Final Concentration | | | Primers: |
| 10x thermopol reaction buffer | 2,5 | 1X | | | f_Gbs_CYC |
| 10 mM dNTPs | 0,5 | 200 µM | | | r_Gbs_CYC |
| 10 µM Forward Primer | 0,5 | 0.2 µM | | | |
| 10 µM Reverse Primer | 0,5 | 0.2 µM | | | |
| Template (colony) | 0 | 5 ng | | | DNA Template: |
| DMSO (optional) | 0 | 3 % | | | Colonies |
| Taq polymerase | 0,125 | 1.0 units/50 µl PCR | | | |
| Nuclease-free Water | 20,875 | | | | |
| Total volume | 25 | | | | |
| | | | | | |
| Number of reactions (including controls): | 22 | | | | |
| | | | | | |
| WORK ON ICE | | | | | |
| Mastermix | | | | | |
| 10x Thermopol reaction buffer | 55 | | | Lane : | Sample : |
| 10 mM dNTPs | 11 | | | | 1 Ladder |
| 10 µM Forward primer | 11 | | | | 2 CYC1-col1-A |
| 10 µM Reverse primer | 11 | | | | 3 CYC1-col2-A |
| DMSO (optional) | 0 | | | | 4 X |
| Phusion DNA Polymerase (ADDED LAST) | 2,75 | | | | 5 X |
| Nuclease-free Water | 459,25 | | | | 6 CYC2-col2-A |
| | | | | | 7 CYC2-col3-A |
| Distribute 24 µl in tubes | | | | | 8 CYC3-col1-A |
| | | | | | 9 CYC3-col2-A |
| | | | | | 10 CYC3-col3-A |
| PCR programming: programe A 'Y-COL- | | | | | 11 Self ligation A |
| Initial Denaturation | 95°C | 6 minutes | | | 12 X |
| | 95°C | 20 sec | | | |
| | 62°C | 30 sec | | Bottom gel | |
| 35 Cycles | 68°C | 1 minute | | | 1 Ladder |
| Final Extension | 72°C | 5 minutes | | | 2 CYC1-col1-B |
| Hold | 4°C | hold | | | 3 CYC1-col2-B |
| | | | | | 4 CYC1-col3-B |
| | | | | | 5 CYC2-col1-B |
| | | | | | 6 CYC2-col2-B |
| | | | | | 7 CYC2-col3-B |
| PCR programming: programe B 'Y-COL- | | | | | 8 CYC3-col1-B |
| Initial Denaturation | 95°C | 7 minutes | | | 9 CYC3-col2-B |
| | 95°C | 30 sec | | | 10 CYC3-col3-B |
| | 62°C | 1 minute | | | 11 Self ligation B |
| 35 Cycles | 67°C | 1 minute 15 | | | 12 Negative PCR control |
| Final Extension | 72°C | 5 minutes | | | |
| Hold | 4°C | hold | | | |